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Signal Transducers and Activators of Transcription as Targets for Small Organic Molecules

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Signal transducers and activators of transcription (STATs) are a family of transcription factors that are of central importance for cellular signaling and have therefore emerged as attractive target proteins for cell-permeable small molecules. This review outlines the basic concept of STAT signaling, the relevance of individual members of the STAT family for cellular signaling and human disease, and generally applicable approaches taken to the identification of small-molecule inhibitors of STATs.

Introduction

Signal transducers and activators of transcription (STATs) are a family of transcription factors that transduce signals from the cell surface to the nucleus.[1,2] The seven STAT family members identified to date display the following common features: 1) an amino-terminal (N) domain involved in protein–protein interactions, including those that lead to the association of two DNA-bound STAT dimers to form tetramers; 2) a coiled-coil-domain that mediates additional interactions with other proteins; 3) a DNA binding domain; 4) a linker domain; 5) a Src homology 2 (SH2) domain for binding of STATs to activated receptors and for dimerization; and 6) a transactivation domain at the C terminus. All STATs contain a conserved tyrosine (Y) between the SH2 domain and the transactivation domain, and, with the exception of STAT2, are known to contain a serine (S) phosphorylation site within the transactivation domain (Figure 1 A).

STATs bind to activated cytokine receptors or growth factor receptors via their SH2 domains (Figure 1 B). Upon ligand-induced receptor dimerization, receptor-associated Janus kinases (JAKs) phosphorylate the cytoplasmic tail of cytokine receptors to create binding sites for the SH2 domain of STATs. Receptor-bound STATs are subsequently phosphorylated at the conserved tyrosine residue C-terminal of the SH2 domain by JAKs or other cytoplasmic tyrosine kinases. Growth factor receptors with intrinsic tyrosine kinase activity can also phosphorylate STATs directly. In addition, STATs can be phosphorylated by activated Src or Abl in the absence of ligand-induced receptor signaling. Tyrosine phosphorylation of STATs induces their dimerization by reciprocal phosphotyrosine–SH2 domain interactions; STAT dimers subsequently translocate to the nucleus, where they regulate gene expression upon binding to specific DNA sequences. Thus, the intracellular localization of STATs depends on their activation state, which is why STATs are often referred to as "latent cyto-

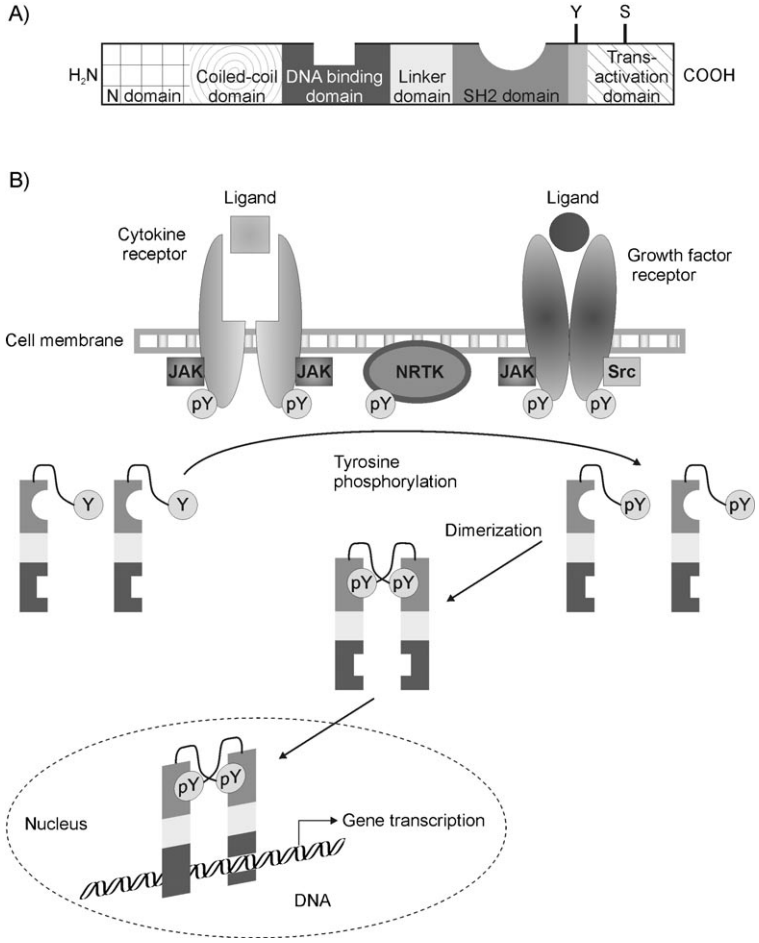

Figure 1. Overview on STATs. A) General structure of STAT proteins. B) Simplified model of signal transduction via STATs. Color codes used for the STAT protein domains are as in A), except that the N domain, the coiled coil domain, and the transactivation domain are omitted for clarity. In part adapted from ref. [79], with permission. Copyright 2006, Elsevier.

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plasmic" transcription factors. At least in the case of STAT1, STAT3, and STAT4, full transcriptional potential requires phosphorylation of the conserved serine residue in the transactivation domain.[3,4] Furthermore, pairwise association of STAT dimers bound to adjacent sites on DNA via their amino-terminal domains increases STAT DNA binding[5] and thereby allows for more efficient transcription from certain promoters.[6,7]

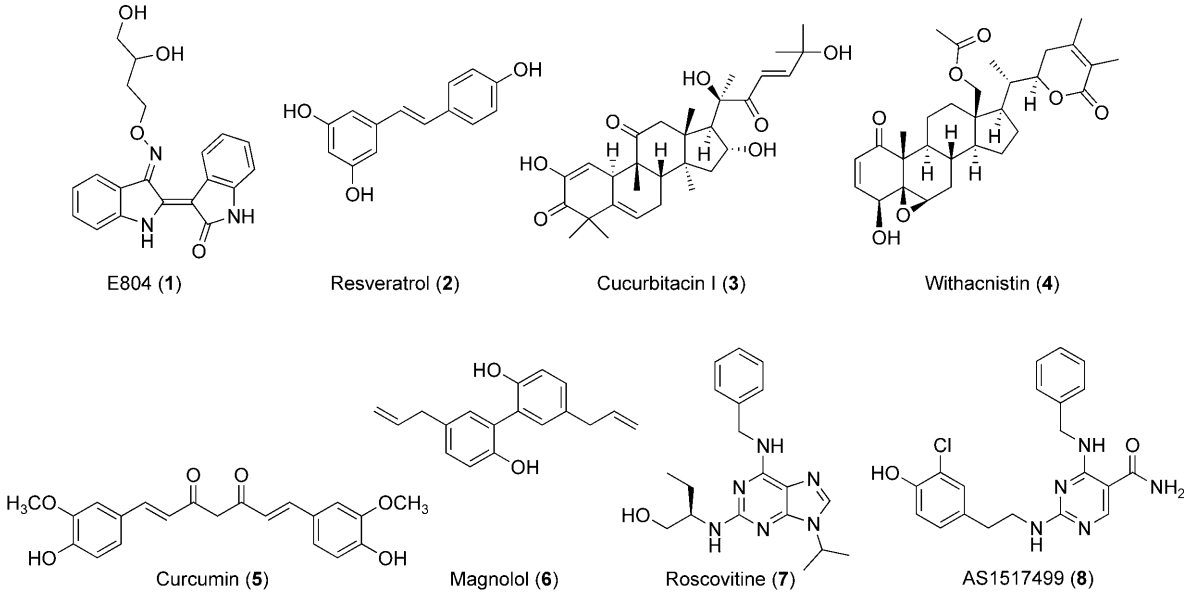
All of the seven STAT proteins known to date are thought to be associated with human disease. STAT1 and STAT2 were reported as DNA binding factors induced by cellular stimulation with interferons (IFNs).[8–10] STAT1 mediates responses to type I and type II IFNs and thus is essential for fighting viral and bacterial infections.[11–13] Similarly, STAT2 is also involved in the biological response to type I IFNs. Since aberrant IFN-mediated signaling leads to inflammatory diseases, STAT1 and STAT2 are putative targets for therapeutic intervention in inflammatory disorders.[12] In addition, STAT1 has been assigned antiproliferative properties and is thought to act as a tumor suppressor.[14] The elevated levels of STAT1 phosphorylation found in tumors are thought to be a cellular defense mechanism against oncogenic transformation mediated by constitutively activated STAT3, which is found in a broad spectrum of human tumors and cancer cell lines.[15] As inhibition of signaling via STAT3 in these cells by a dominant negative mutant,[16,17] antisense approaches,[18] decoy oligonucleotides,[19–21] siRNAs,[22–24] peptide aptamers,[25,26] and G-quartet oligonucleotides[27,28] has been demonstrated to suppress tumor growth and to induce apoptosis in cancer cells, STAT3 is regarded as a strong candidate target for cancer therapy[29–36] (see ref. [36] for a concomitant review on STAT3 inhibitors published in our sister journal *ChemMedChem*). In contrast to STAT1, STAT3 can exert both pro- and anti-inflammatory functions.[12,37] STAT4 mediates responses to proinflammatory cytokines which initiate and stabilize T helper (Th) lymphocytes class 1-mediated cytokine production.[12,38,39] Inhibition of STAT4 signaling with antisense oligonucleotides was shown to suppress the development of collagen-induced arthritis in a mouse model, and STAT4 is thus considered to be a potential target for treating chronic arthritis.[39] Similar to STAT3, the STAT5 isoforms STAT5a and STAT5b (93% identity at the protein level) are overactive in several kinds of human tumors, including leukemias, breast cancer, uterine cancer, prostate cancer, and squamous cell carcinoma of the head and neck (SCCHN).[40] Inhibition of signaling via STAT5, especially STAT5b, has been shown to inhibit tumor growth and to induce apoptosis of tumor cells.[41–43] Finally, STAT6 mediates signaling by IL-4 and IL-13 and thereby assumes a crucial role in asthma pathogenesis.[44]

Approaches towards the Inhibition of STATs

Small-molecule inhibitors with isoform selectivity for a single STAT could be valuable tools to clarify the complex biological activities of STAT family members in genetically unmodified systems. Inhibitors of STAT signaling can be categorized into agents which act via a direct or via an indirect mode.[45] Indirect small-molecule inhibitors of STATs, which are also referred to as STAT signaling inhibitors, do not interact with the STAT(s), but modulate the activity of a biomolecule that in turn has a regulatory function for the STAT(s) of interest. In contrast, direct inhibitors of STATs physically interact with them and thereby interfere with their ability to regulate transcription.

Indirect Inhibitors of STAT Signaling

This group includes inhibitors of the enzymatic activities of those tyrosine kinases which activate STATs on the conserved tyrosine residue between the SH2 domain and the transactivation domain. The alkylated indirubin oxime E804 (**1**; Scheme 1) was shown to inhibit STAT3 signaling in breast cancer cells by inhibiting upstream kinase activity, presumably that of c-Src.[46] Indirubin itself is a constituent of a Chinese herbal prescription used for treatment of chronic myelogenous leukemia[47] and a



Scheme 1. Examples of indirect inhibitors of STATs.

known inhibitor of cyclin-dependent kinases.[48] A similar mechanism of action was suggested for the natural product Resveratrol (**2**).[49] Other compounds inhibit STAT3 phosphorylation by unknown or speculative mechanisms. Cucurbitacin I (JSI-124; **3**)[50] and other cucurbitacin family members[51] were shown to inhibit signaling via STAT3. The identity of the STAT3 signaling inhibitor NSC 135075, which is part of the National Cancer Institute (NCI) library, had originally been reported to the researchers as cucurbitacin Q,[51] but was only recently corrected by the NCI to withacnistin (**4**).[52] Curcumin (**5**), another indirect natural product inhibitor of STAT3 signaling,[53] has also been identified as an inhibitor of numerous additional signaling pathways.[54] Similarly, magnolol (**6**) was shown to inhibit signaling via STAT3,[55] but also via NF-κB.[56,57] Roscovitine (**7**), an inhibitor of cyclin-dependent kinases, was found to inhibit STAT5 phosphorylation.[42] High-throughput screening of chemical libraries in a STAT6 reporter assay and subsequent chemical development led to the discovery of a series of substituted aminopyrimidine-5-carboxamides as STAT6 signaling inhibitors. The most potent compound dubbed AS1517499 (**8**) inhibited IL-4 dependent transcription, which is mediated by STAT6, and selectively inhibited IL-4-induced Th2 differentiation of mouse spleen T cells in the low nanomolar concentration range.[58] Based on the published data, it cannot be excluded that **8** inhibits STAT6 directly; however, data indicating a direct interaction between **8** and STAT6 were not provided.

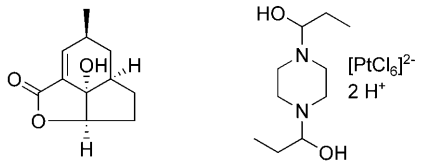
These examples illustrate that indirect inhibitors of STATs can effectively inhibit STAT activation and exert potent biological effects.[59] However, targeting an upstream regulatory molecule is generally an unsatisfactory means by which to investigate the precise function of a signaling molecule, as cross-talk between signaling pathways is common. Thus, indirect inhibitors of STAT signaling are usually not suited as molecular research tools to clarify the relevance of a given STAT protein for a biological process.

Direct Inhibitors of STATs

Direct inhibitors with selectivity for a STAT isoform can generally be categorized into three groups according to their mechanisms of action: inhibitors of the function of the STAT DNA binding domain, of the STAT SH2 domain, and of the STAT amino-terminal domain.

Inhibition of STATs by Blocking of their DNA Binding Domains

To date, this approach has only been applied to the inhibition of STAT3. As an example for this approach, the natural product galiellalactone (**9**, Scheme 2), originally reported as a weak inhibitor of the de novo synthesis of α-amylases, proteases, and phosphatases in embryoless halves of wheat seeds,[60] was also found to inhibit interleukin (IL)-6-mediated STAT3 signaling.[61] The absolute configuration of the natural product was only recently determined by chemical synthesis.[62,63] As galiellalactone inhibited DNA binding of activated STAT3 without affecting STAT3 tyrosine phosphorylation, the compound was assumed



Galiellalactone (**9**) IS3 295 (**10**)

Scheme 2. Inhibitors of STAT3 DNA binding.

to bind to the DNA binding domain of dimeric STAT3, possibly by covalently modifying a cysteine residue in the STAT3 DNA binding domain. The platinum complex IS3 295 (NSC 295558; **10**) was shown to block DNA binding of STAT3 by binding to the protein, and to inhibit STAT3 functions in tumor cells harboring constitutive STAT3 activation, thereby inducing cell-cycle arrest and apoptosis.[64]

Inhibition of STATs by Blocking of their SH2 Domains

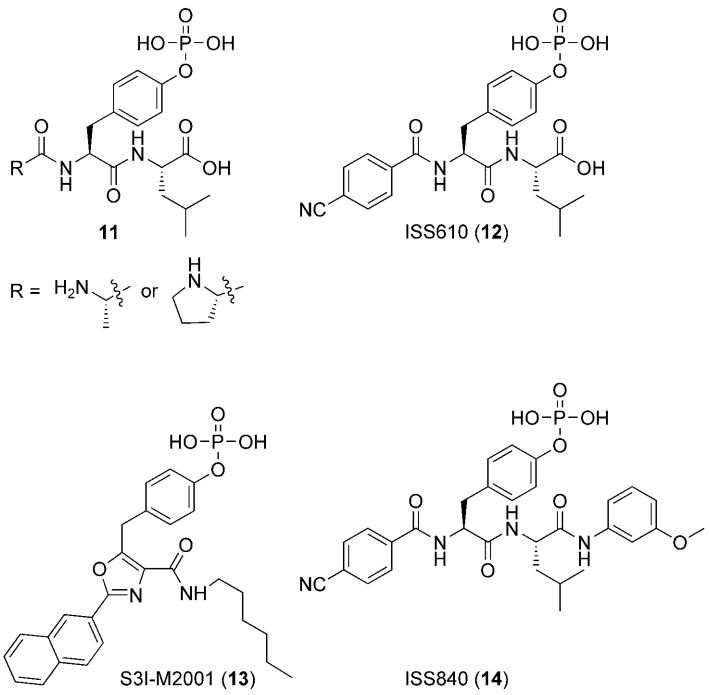
Since the SH2 domain is required for both tyrosine-phosphorylation and dimerization of STATs, an effective approach which would allow for targeting of only a single STAT is the inhibition of the function of its SH2 domain.[65] This should not only inhibit STAT activation, but also prevent dimerization of any STAT molecules which escape inhibition of activation (Figure 1 B).

Peptide-Based Inhibitors of STAT SH2 Domains

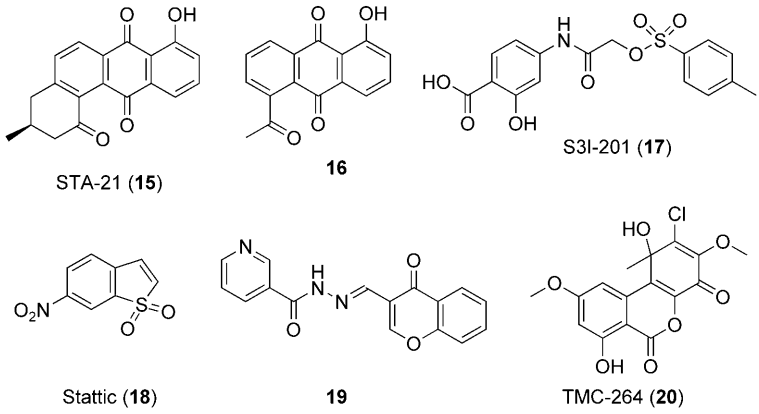
The feasibility of inhibiting activation of members of the STAT family with a ligand for their SH2 domains was demonstrated for STAT3[65–69] and STAT6.[70] A cell-permeable fusion peptide comprising the sequence GASSGEEGpYKPFQDLC derived from the interleukin (IL)-4 receptor was shown to inhibit IL-4-dependent STAT6 phosphorylation and STAT6-dependent transcription.[70] Data generated by the application of a cell-permeable fusion peptide comprising the STAT6-derived sequence GRGpYVSTT, which was known to bind to the STAT6 SH2 domain, in mouse models suggested the inhibition of the STAT6 SH2 domain as a therapeutic approach for the treatment of allergic rhinitis and asthma.[71]

The vast majority of studies targeting a STAT SH2 domain have been performed on STAT3. A fusion peptide between the hexapeptide PpYLKTK (the motif which mediates STAT3 dimerization) and a membrane translocating sequence was shown to inhibit STAT3 tyrosine phosphorylation, STAT3-dependent gene transcription, and oncogenic transformation.[66] The tripeptide motif A/PpYL (**11**, Scheme 3) was shown to be sufficient for inhibition of STAT3 dimerization in vitro, and served as the starting point for the design of peptide mimetics with reduced peptidic character. Initially, this led to the generation of the peptide mimetic ISS 610 (**12**), which was shown to inhibit DNA binding of activated STAT3 with seven- to eightfold preference over STAT1, and to display STAT3-dependent effects in tissue culture.[72] Both the cell-permeable fusion peptide comprising the PpYLKTK motif and ISS 610 require their use at high con-

centrations in tissue culture (500–1000 μM), probably due to their peptidic nature and the presence of a phosphotyrosine residue, which is likely to negatively affect cellular uptake and to render the compounds susceptible to phosphatases. Analysis of binding between ISS610 and the STAT3 SH2 domain[73] as suggested by computational modeling led to the design of the oxazole-based peptide mimetic S3I-M2001 (**13**), which is probably the STAT3 SH2 domain-directed peptide mimetic with the least peptidic character described so far.[74] The compound has only a single remaining peptide bond, but still retains the side chain of the central phosphotyrosine. Nevertheless, S3I-M2001 displayed strong STAT3-dependent activity in cellular assays at 30–100 μM, which is a significant improvement over previous peptide-based ligands to the STAT3 SH2 domain. S3I-M2001 disrupted tyrosine phosphorylated STAT3, and inhibited STAT3-mediated gene transcription, malignant transformation, survival, and migration. Moreover, the compound was shown to inhibit proliferation of a breast cancer cell line harboring constitutive STAT3 activation in a mouse xenograft model. Interestingly, introduction of a *m*-methoxyaniline group at the carboxy terminus of ISS 610 was shown to inverse the specificity of the peptide mimetic for STAT3 over STAT1; the resulting compound ISS840 (**14**) displayed a 20-fold preference for disruption of activated STAT1 dimers over STAT3 and is currently the STAT SH2 domain-directed agent with the strongest preference for STAT1 over STAT3.[75]



Scheme 3. Peptide-based inhibitors of STAT SH2 domains.



Scheme 4. Non-peptidic inhibitors of STAT SH2 domains.

Nonpeptidic Inhibitors of STAT SH2 Domains

The availability of the crystal structure of DNA bound, tyrosine phosphorylated STAT3[73] permitted two independent studies which applied virtual screening of chemical databases for the identification of nonpeptidic inhibitors of the STAT3 SH2 domain.[76,77] Screening of chemical databases identified candidate compounds with an increased likelihood of binding to the STAT3 SH2 domain. Molecules which could be docked in the STAT3 SH2 domain were subsequently tested in actual biochemical assays. The first compound discovered by this route, STA-21 (NSC 628869; **15**; Scheme 4), was shown to inhibit DNA-binding of prephosphorylated STAT3, and to display STAT3-dependent cellular effects.[76] Recently, the STA-21 derivative **16** with similar activity was reported, which is more amenable to structural modification.[78] Furthermore, the compound S3I-201 (NSC 74859; **17**) was modeled into the STAT3 SH2 domain and was shown to inhibit STAT3 dimerization. The compound inhibited STAT3-mediated gene expression, induced apoptosis in cells with constitutively activated STAT3, and inhibited tumor growth in a mouse xenograft model.

The feasibility of identifying small-molecule inhibitors of the STAT3 SH2 domain by biochemical screening was demonstrated by the identification and characterization of Stattic (**18**).[79] This compound was discovered in an in vitro assay based on fluorescence polarization which analyzes the effect of test compounds on the function of the STAT3 SH2 domain.[80] Stattic was found to inhibit the function of the SH2 domain of both unphosphorylated and phosphorylated STAT3, and to display a preference for STAT3 over the family members STAT1 and STAT5b in vitro. Furthermore, Stattic was shown to inhibit nuclear translocation of STAT3 with good selectivity over STAT1 in a hepatocellular carcinoma cell line, and selectively increased the apoptotic rate of breast cancer cell lines harboring constitutive STAT3 activity.

The application of this screening approach to STAT5b allowed for the discovery of the first reported inhibitors of the function of the STAT5 SH2 domain. Chromone-based acyl hydrazone **19** and similar compounds were shown to selectively inhibit the function of the STAT5b SH2 domain in a fluorescence polarization assay.[81] Importantly for chemical biology studies, the compounds allowed for the inhibition of IFN-α mediated activation of STAT5 with good selectivity over the activation of STAT3 and STAT1.[82] The chromone ring system, which is found in numerous biologically active natural products, was demonstrated to be important for the compounds' inhibitory activities.

The tricyclic heptaketide TMC-264 (**20**) from the fungus *Phoma* sp. *TC 1674* was found in a screen of microbial extracts.[83] TMC-264 was shown to inhibit IL-4-induced gene transcription, which is mediated by STAT6, with good selectivity over IFN-γ-induced gene transcription, which is mediated by STAT1.[84] Mechanistic analysis revealed that TMC-264 blocked tyrosine phosphorylation of STAT6 with good selectivity over tyrosine phosphorylation of STAT1 and STAT5. Furthermore, the compound inhibited DNA binding of phosphorylated STAT6, but not of phosphorylated STAT1. This activity profile is consistent with a model by which TMC-264 selectively inhibits the function of the STAT6 SH2 domain, even though the original publication[84] does not explicitly propose this mechanism of action.

Inhibitors of STAT N Domains

Even though it has been known for a number of years that pairwise association of phosphorylated STAT dimers bound to adjacent DNA sites via their amino-terminal (N) domains can increase their transcriptional potential,[6,7] the concept of targeting the N domain of a STAT family member has only very recently been explored. The STAT N domains consist of eight helices, and are highly conserved amongst STAT family members.[85] A peptide comprising helix two of the STAT4 N domain was found to cause significant structural changes to the full-length STAT4 N domain.[86] Based on the STAT4-derived peptide sequence used in the NMR experiments, a small library of corresponding STAT3-derived peptides comprising helix two of the STAT3 N domain and peptide sequences which confer cell permeability was synthesized. Amongst others, a peptide comprising the motif LDTRYLEQLHKLY fused to penetratin was shown to bind to full-length STAT3 with good selectivity over STAT1 in cells, and to induce apoptotic death of cancer cell lines in a STAT3-dependent manner. These data provide proof of principle that targeting the STAT N domain is a valid approach by which to interfere with STAT activity.

Outlook

The central role of members of the STAT transcription factor family in disease-related processes makes them highly desirable targets for the development of cell-permeable functional modulators of their activities. The knowledge of the function and structure of STATs gained in recent years has allowed the initiation of small-molecule discovery programs aimed at identifying potent and specific inhibitors of STAT functions. Such cell-permeable inhibitors will be helpful in clarifying the role of these central regulators of key biological processes, in confirming or disproving the relevance of a given STAT in disease models, and should have the potential to stimulate medicinal chemistry efforts aimed at finding small molecules with sufficient potencies to serve as drugs for human use. The impressive achievements related to the discovery of effective and selective agents targeting members of the STAT family demonstrate the potential of interdisciplinary research, integrating synthetic organic chemistry, biochemistry, and cell biology. Contrary to the common perception that transcription factors are not amenable to functional modulation by cell-permeable molecules, STATs, like other transcription factors, are emerging as targets for small organic molecules.[87–89]

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[1] J. E. Darnell Jr, *Nat. Rev. Cancer* **2002**, *2*, 740–749.
[2] C. Schindler, D. E. Levy, T. Decker, *J. Biol. Chem.* **2007**, *282*, 20059–20063.
[3] D. E. Levy, J. E. Darnell Jr, *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 651–662.
[4] C. M. Horvath, *Trends Biochem. Sci.* **2000**, *25*, 496–502.
[5] U. Vinkemeier, S. L. Cohen, I. Moarefi, B. T. Chait, J. Kuriyan, J. E. Darnell, Jr., *EMBO J.* **1996**, *15*, 5616–5626.
[6] X. Zhang, J. E. Darnell Jr. , *J. Biol. Chem.* **2001**, *276*, 33576–33581.
[7] S. John, U. Vinkemeier, E. Soldaini, J. E. Darnell, Jr., W. J. Leonard, *Mol. Cell. Biol.* **1999**, *19*, 1910–1918.
[8] K. Shuai, C. Schindler, V. R. Prezioso, J. E. Darnell Jr. , *Science* **1992**, *258*, 1808–1812.
[9] C. Schindler, K. Shuai, V. R. Prezioso, J. E. Darnell Jr. , *Science* **1992**, *257*, 809–813.
[10] J. E. Darnell Jr. , I. M. Kerr, G. R. Stark, *Science* **1994**, *264*, 1415–1421.
[11] M. G. Katze, Y. He, M. Gale Jr. , *Nat. Rev. Immunol.* **2002**, *2*, 675–687.
[12] E. Pfitzner, S. Kliem, D. Baus, C. M. Litterst, *Curr. Pharm. Des.* **2004**, *10*, 2839–2850.
[13] T. Decker, S. Stockinger, M. Karaghiosoff, M. Müller, P. Kovarik, *J. Clin. Invest.* **2002**, *109*, 1271–1277.
[14] D. H. Kaplan, V. Shankaran, A. S. Dighe, E. Stockert, M. Aguet, L. J. Old, R. D. Schreiber, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 7556–7561.
[15] R. Buettner, L. B. Mora, R. Jove, *Clin. Cancer Res.* **2002**, *8*, 945–954.
[16] G. Niu, R. Heller, R. Catlett-Falcone, D. Coppola, M. Jaroszeski, W. Dalton, R. Jove, H. Yu, *Cancer Res.* **1999**, *59*, 5059–5063.
[17] R. Catlett-Falcone, T. H. Landowski, M. Oshiro, J. Turkson, A. Levitzki, R. Savino, G. Ciliberto, L. Moscinski, J. L. Fernandez-Luna, G. Nunez, W. S. Dalton, R. Jove, *Immunity* **1999**, *10*, 105–115.
[18] M. S. Redell, D. J. Tweardy, *Curr. Pharm. Des.* **2005**, *11*, 2873–2887.
[19] S. Xi, W. E. Gooding, J. R. Grandis, *Oncogene* **2005**, *24*, 970–979.

[20] P. L. Leong, G. A. Andrews, D. E. Johnson, K. F. Dyer, S. Xi, J. C. Mai, P. D. Robbins, S. Gadiparthi, N. A. Burke, S. F. Watkins, J. R. Grandis, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4138–4143.
[21] B. E. Barton, T. F. Murphy, P. Shu, H. F. Huang, M. Meyenhofer, A. Barton, *Mol. Cancer Ther.* **2004**, *3*, 1183–1191.
[22] L. Konnikova, M. Kotecki, M. M. Kruger, B. H. Cochran, *BMC Cancer* **2003**, *3*, 23.
[23] X. Ling, R. B. Arlinghaus, *Cancer Res.* **2005**, *65*, 2532–2536.
[24] L. Gao, L. Zhang, J. Hu, F. Li, Y. Shao, D. Zhao, D. V. Kalvakolanu, D. J. Kopecko, X. Zhao, D. Q. Xu, *Clin. Cancer Res.* **2005**, *11*, 6333–6341.
[25] C. Borghouts, C. Kunz, N. Delis, B. Groner, *Mol. Cancer Res.* **2008**, *6*, 267–281.
[26] K. Nagel-Wolfrum, C. Buerger, I. Wittig, K. Butz, F. Hoppe-Seyler, B. Groner, *Mol. Cancer Res.* **2004**, *2*, 170–182.
[27] N. Jing, Y. Li, W. Xiong, W. Sha, L. Jing, D. J. Tweardy, *Cancer Res.* **2004**, *64*, 6603–6609.
[28] N. Jing, Q. Zhu, P. Yuan, Y. Li, L. Mao, D. J. Tweardy, *Mol. Cancer Ther.* **2006**, *5*, 279–286.
[29] B. B. Aggarwal, G. Sethi, K. S. Ahn, S. K. Sandur, M. K. Pandey, A. B. Kunnumakkara, B. Sung, H. Ichikawa, *Ann. N. Y. Acad. Sci.* **2006**, *1091*, 151–169.
[30] S. Desrivières, C. Kunz, I. Barash, V. Vafaizadeh, C. Borghouts, B. Groner, *J. Mammary Gland Biol. Neoplasia* **2006**, *11*, 75–87.
[31] J. E. Darnell, *Nat. Med.* **2005**, *11*, 595–596.
[32] L. Klampfer, *Curr. Cancer Drug Targets* **2006**, *6*, 107–121.
[33] R. J. Leeman, V. W. Lui, J. R. Grandis, *Expert Opin. Biol. Ther.* **2006**, *6*, 231–241.
[34] J. Deng, F. Grande, N. Neamati, *Curr. Cancer Drug Targets* **2007**, *7*, 91–107.
[35] K. Al Zaid Siddiquee, J. Turkson, *Cell Regul.* **2008**, *18*, 254–267.
[36] S. Fletcher, J. Turkson, P. T. Gunning, *ChemMedChem* **2008**, *3*, 1159–1168.
[37] D. E. Levy, C.-K. Lee, *J. Clin. Invest.* **2002**, *109*, 1143–1148.
[38] M. H. Kaplan, *Immunol. Res.* **2005**, *31*, 231–242.
[39] K. M. Hildner, P. Schirmacher, I. Atreya, M. Dittmayer, B. Bartsch, P. R. Galle, S. Wirtz, M. F. Neurath, *J. Immunol.* **2007**, *178*, 3427–3436.
[40] I. Wittig, B. Groner, *Curr. Cancer Drug Targets* **2005**, *5*, 449–463.
[41] S. Xi, Q. Zhang, W. E. Gooding, T. E. Smithgall, J. R. Grandis, *Cancer Res.* **2003**, *63*, 6763–6771.
[42] S. Mohapatra, B. Chu, S. Wei, J. Djeu, P. K. Epling-Burnette, T. Loughran, R. Jove, W. J. Pledger, *Cancer Res.* **2003**, *63*, 8523–8530.
[43] J. B. Demoulin, C. Uyttenhove, D. Lejeune, A. Mui, B. Groner, J. C. Renauld, *Cancer Res.* **2000**, *60*, 3971–3977.
[44] D. Hebenstreit, G. Wirnsberger, J. Horejs-Hoeck, A. Duschl, *Cytokine Growth Factor Rev.* **2006**, *17*, 173–188.
[45] J. S. McMurray, *Chem. Biol.* **2006**, *13*, 1123–1124.
[46] S. Nam, R. Buettner, J. Turkson, D. Kim, J. Q. Cheng, S. Muehlbeyer, F. Hippe, S. Vatter, K. H. Merz, G. Eisenbrand, R. Jove, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5998–6003.
[47] Z. Xiao, Y. Hao, B. Liu, L. Qian, *Leuk Lymphoma* **2002**, *43*, 1763–1768.
[48] G. Eisenbrand, F. Hippe, S. Jakobs, S. Muehlbeyer, *J. Cancer Res. Clin. Oncol.* **2004**, *130*, 627–635.
[49] A. Kotha, M. Sekharam, L. Cilenti, K. Siddiquee, A. Khaled, A. S. Zervos, B. Carter, J. Turkson, R. Jove, *Mol. Cancer Ther.* **2006**, *5*, 621–629.
[50] M. A. Blaskovich, J. Sun, A. Cantor, J. Turkson, R. Jove, S. M. Sebti, *Cancer Res.* **2003**, *63*, 1270–1279.
[51] J. Sun, M. A. Blaskovich, R. Jove, S. K. Livingston, D. Coppola, S. M. Sebti, *Oncogene* **2005**, *24*, 3236–3245.
[52] J. Sun, M. A. Blaskovich, R. Jove, S. K. Livingston, D. Coppola, S. M. Sebti, *Oncogene* **2008**, *27*, 1344.
[53] A. C. Bharti, N. Donato, B. B. Aggarwal, *J. Immunol.* **2003**, *171*, 3863–3871.
[54] B. B. Aggarwal, A. Kumar, A. C. Bharti, *Anticancer Res.* **2003**, *23*, 363–398.
[55] S. C. Chen, Y. L. Chang, D. L. Wang, J. J. Cheng, *Br. J. Pharmacol.* **2006**, *148*, 226–232.
[56] Y. H. Chen, S. J. Lin, J. W. Chen, H. H. Ku, Y. L. Chen, *Br. J. Pharmacol.* **2002**, *135*, 37–47.
[57] J. Lee, E. Jung, J. Park, K. Jung, S. Lee, S. Hong, E. Park, J. Kim, S. Park, D. Park, *Planta Med.* **2005**, *71*, 338–343.
[58] S. Nagashima, M. Yokota, E. Nakai, S. Kuromitsu, K. Ohga, M. Takeuchi, S. Tsukamoto, M. Ohta, *Bioorg. Med. Chem.* **2007**, *15*, 1044–1055.
[59] A. Iwamaru, S. Szymanski, E. Iwado, H. Aoki, T. Yokoyama, I. Fokt, K. Hess, C. Conrad, T. Madden, R. Sawaya, S. Kondo, W. Priebe, Y. Kondo, *Oncogene* **2007**, *26*, 2435–2444.
[60] R. Hautzel, H. Anke, *Z. Naturforsch. C* **1990**, *45*, 1093–1098.
[61] M. Weidler, J. Rether, T. Anke, G. Erkel, *FEBS Lett.* **2000**, *484*, 1–6.
[62] M. Johansson, O. Sterner, *J. Antibiot.* **2002**, *55*, 663–665.
[63] M. Johansson, O. Sterner, *Org. Lett.* **2001**, *3*, 2843–2845.
[64] J. Turkson, S. Zhang, L. B. Mora, A. Burns, S. Sebti, R. Jove, *J. Biol. Chem.* **2005**, *280*, 32979–32988.
[65] D. R. Coleman, Z. Ren, P. K. Mandal, A. G. Cameron, G. A. Dyer, S. Muranjan, M. Campbell, X. Chen, J. S. McMurray, *J. Med. Chem.* **2005**, *48*, 6661–6670.
[66] J. Turkson, D. Ryan, J. S. Kim, Y. Zhang, Z. Chen, E. Haura, A. Laudano, S. Sebti, A. D. Hamilton, R. Jove, *J. Biol. Chem.* **2001**, *276*, 45443–45455.
[67] Z. Ren, L. A. Cabell, T. S. Schaefer, J. S. McMurray, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 633–636.
[68] P. K. Mandal, P. A. Heard, Z. Ren, X. Chen, J. S. McMurray, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 654–656.
[69] J. Chen, Z. Nikolovska-Coleska, C. Y. Yang, C. Gomez, W. Gao, K. Krajewski, S. Jiang, P. Roller, S. Wang, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3939–3942.
[70] S. Stolzenberger, M. Haake, A. Duschl, *Eur. J. Biochem.* **2001**, *268*, 4809–4814.
[71] C. T. McCusker, Y. Wang, J. Shan, M. W. Kinyanjui, A. Villeneuve, H. Michael, E. D. Fixman, *J. Immunol.* **2007**, *179*, 2556–2564.
[72] J. Turkson, J. S. Kim, S. Zhang, J. Yuan, M. Huang, M. Glenn, E. Haura, S. Sebti, A. D. Hamilton, R. Jove, *Mol. Cancer Ther.* **2004**, *3*, 261–269.
[73] S. Becker, B. Groner, C. W. Muller, *Nature* **1998**, *394*, 145–151.
[74] K. A. Siddiquee, P. T. Gunning, M. Glenn, W. P. Katt, S. Zhang, C. Schroeck, S. M. Sebti, R. Jove, A. D. Hamilton, J. Turkson, *ACS Chem. Biol.* **2007**, *2*, 787–798.
[75] P. T. Gunning, W. P. Katt, M. Glenn, K. Siddiquee, J. S. Kim, R. Jove, S. M. Sebti, J. Turkson, A. D. Hamilton, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1875–1878.
[76] H. Song, R. Wang, S. Wang, J. Lin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4700–4705.
[77] K. Siddiquee, S. Zhang, W. C. Guida, M. A. Blaskovich, B. Greedy, H. R. Lawrence, M. L. Yip, R. Jove, M. M. McLaughlin, N. J. Lawrence, S. M. Sebti, J. Turkson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7391–7396.
[78] D. Bhasin, K. Cisek, T. Pandharkar, N. Regan, C. Li, B. Pandit, J. Lin, P. K. Li, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 391–395.
[79] J. Schust, B. Sperl, A. Hollis, T. U. Mayer, T. Berg, *Chem. Biol.* **2006**, *13*, 1235–1242.
[80] J. Schust, T. Berg, *Anal. Biochem.* **2004**, *330*, 114–118.
[81] J. Muller, J. Schust, T. Berg, *Anal. Biochem.* **2008**, *375*, 249–254.
[82] J. Müller, B. Sperl, W. Reindl, A. Kiessling, T. Berg, *ChemBioChem* **2008**, *9*, 723–727.
[83] M. Sakurai, M. Nishio, K. Yamamoto, T. Okuda, K. Kawano, T. Ohnuki, *Org. Lett.* **2003**, *5*, 1083–1085.
[84] M. Sakurai, M. Nishio, K. Yamamoto, T. Okuda, K. Kawano, T. Ohnuki, *J. Antibiot.* **2003**, *56*, 513–519.
[85] U. Vinkemeier, I. Moarefi, J. E. Darnell, Jr., J. Kuriyan, *Science* **1998**, *279*, 1048–1052.
[86] O. A. Timofeeva, V. Gaponenko, S. J. Lockett, S. G. Tarasov, S. Jiang, C. J. Michejda, A. O. Perantoni, N. I. Tarasova, *ACS Chem. Biol.* **2007**, *2*, 799–809.
[87] H. D. Arndt, *Angew. Chem.* **2006**, *118*, 4664–4673; *Angew. Chem. Int. Ed.* **2006**, *45*, 4552–4560.
[88] C. Y. Majmudar, A. K. Mapp, *Curr. Opin. Chem. Biol.* **2005**, *9*, 467–474.
[89] T. Berg, *Curr. Opin. Chem. Biol.* **2008**, *12*, DOI: 10.1016/j.cbpa.2008.07.023.

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